Prenatal CMA using exon-level coverage for 'disease gene' CNV interrogations in the era of non-invasive prenatal testing

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BACKGROUND

Non-invasive prenatal testing (NIPT) with cell-free DNA is widely accepted as standard of care to screen for common aneuploidies, and some platforms also include screening for microdeletions/duplications. This shift in clinical practice affects the use of chromosomal microarray analysis (CMA) in the prenatal setting. In addition, microarrays with exon-by-exon coverage for disease genes enable detection of intragenic copy number variants (CNVs) leading to increased sensitivity to postnatal diagnosis but such arrays are rarely used in prenatal CMA.

This study investigated the role of prenatal CMA in the era of NIPT based on a single laboratory's experience.

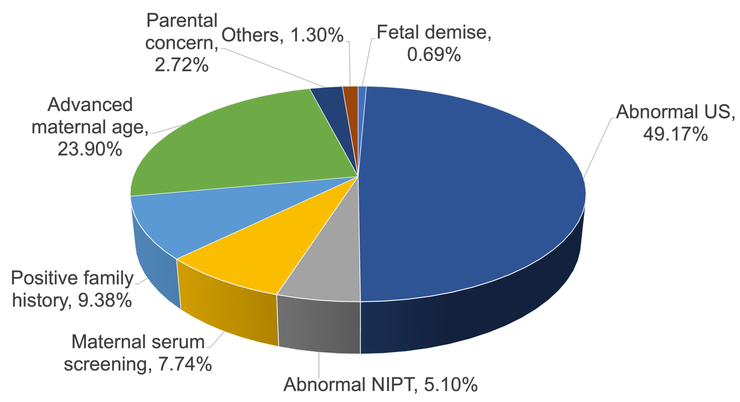
METHODS

- We retrospectively reviewed the results of all amniotic fluid and chorionic villus samples that were analyzed by CMA using custom-designed Agilent arrays during the last 11 years at Baylor Genetics.
- For 80% of samples, CMA was performed using arrays that include exon-by-exon coverage for >1,700 genes.
- Parental samples were received concurrently to evaluate maternal cell contamination and to facilitate data interpretation for most cases.

RESULTS

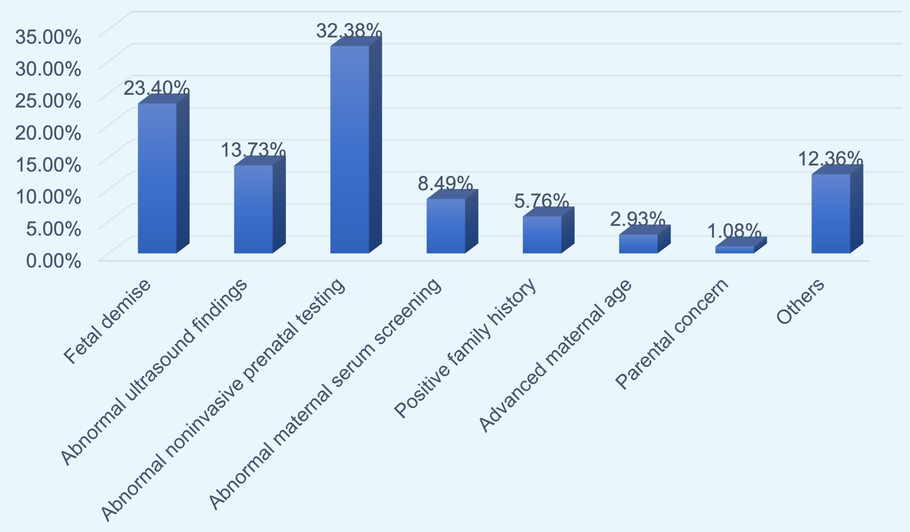
- CMA cases were grouped according to the primary indication. If there were multiple indications, the case was classified based on the primary indication with the highest priority in the following order: fetal demise, abnormal ultrasound findings, abnormal NIPT, positive maternal serum screening, positive family history, advanced maternal age (AMA), parental concern, and others.
- The most common primary indications were abnormal prenatal ultrasound findings (US) and AMA. This is consistent with previous literature (Breman et al. 2012). Abnormal or atypical NIPT was observed in 5.1% of cases, and another 1.0% cases had both an abnormal ultrasound and abnormal NIPT indication (Figure 1).

Figure 1 Distribution of CMA cases by primary indication



- The overall CMA detection rate of clinically significant findings was 10.7% and the diagnostic rate for pregnancies with abnormal ultrasound findings was 13.7%.
- The diagnostic rate was highest (32.4%) for cases with abnormal or atypical NIPT results as the primary indication. Among those, the most frequent NIPT findings were increased risk or positive result for autosomal aneuploidy, followed by increased risk or positive result for sex chromosome aneuploidy, microdeletion/duplication, and inconclusive or nonreportable findings.

Figure 2 Diagnostic rate by primary indication



Cases with increased risk for microdeletion/duplication by NIPT

- Of the 70 cases with increased risk for microdeletion/duplication, CMA confirmed the CNV in 13 (18.6%) of cases (Table 1).
- Of the 32 cases with increased risk of 22q11.2 deletion, the typical 22q11.2 deletion was detected by CMA in four cases, while the other 28 cases showed no copy number changes in this region.
- For the other 9 cases with CMA confirmed CNVs other than 22q deletion, all the CNVs are >10 Mb in size except for one case with a 1.4 Mb maternally inherited duplication in 21q22.2.
- Notably, in one case, while NIPT reported increased risk for 1p36 deletion, CMA did not detect copy number variants in chromosome 1, instead, but CMA showed a terminal deletion in chromosome 3 that is the typical deletion for 3q29 microdeletion syndrome.

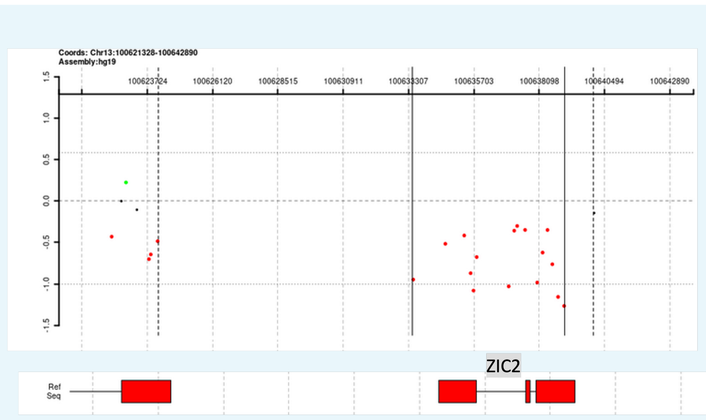
Table 1 Comparison of the CMA results with the NIPT results for the cases with increased risk for deletions/duplications

NIPT showing increased risk	# of cases	# of cases with concordant CMA results	Confirmation rate
22q11.2 deletion	32	4	12.5%
1p36 deletion	8	0	0.0%
15q PWS/AS deletion	8	0	0.0%
5p deletion	6	0	0.0%
18p duplication	3	2	66.7%
Others	13	7	53.8%
Total	70	13	18.6%

Single gene deletions/duplications detected by prenatal CMA

- Prenatal CMA findings include aneuploidy, triploidy, and deletions/duplications not involving an entire chromosome (N=321 cases).
- Due to the increased probe coverage in genes for established rare disease traits, CMA detected clinically significant CNVs affecting single protein coding genes in 18 cases. The genes affected in these cases include *DMD* (N=9 cases), *NRXN1* (N=2 cases), and seven genes in each of one case (*ATP7A, KAL1, MED13L, PAFAH1B1, RPL11, STS,* and *ZIC2*).
- The smallest finding is a de novo ~5.6 Kb deletion of the *ZIC2* gene in a fetus with holoprosencephaly detected by ultrasound (Figure 2). Haploinsufficiency of *ZIC2* causes holoprosencephaly 5 (OMIM # 609637). Holoprosencephaly is a complex brain malformation resulting from incomplete cleavage of the prosencephalon, affecting both the forebrain and the face.

Figure 3 A ~5.6 kb deletion affecting only the *ZIC2* gene was detected



CONCLUSION

- Prenatal CMA remains essential for the detection of microdeletions/duplications because NIPT screening coverage of submicroscopic copy number changes is limited.
- We also show that increased probe coverage of disease genes on prenatal microarrays enables detection of single gene copy number changes.
- Finally, this confirmation data demonstrates the importance of a diagnostic test such as CMA in follow up to NIPT results that are positive or increased risk for microdeletions/duplications.

REFERENCES

- Breman A, Pursley AN, Hixson P, Bi W, Ward P, Bacino CA, Shaw C, Lupski JR, Beaudet A, Patel A, Cheung SW, Van den Veyver I. Prenatal chromosomal microarray analysis in a diagnostic laboratory; experience with >1000 cases and review of the literature. Prenat Diagn. 2012;32(4):351-61. PMID: 22467166.